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IMPACT OF THE WASTEWATER-MIXING ZONE ON ATTENUATION OF PHARMACEUTICALS IN NATURAL WATERS: IMPLICATIONS FOR AN IMPACT ZONE INCLUSIVE ENVIRONMENTAL RISK ASSESSMENT

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Abstract

The direct discharge of untreated wastewater has been identified as an important source of environmental contamination by active pharmaceutical ingredients and other 'down-the-drain' chemicals in developing countries. It necessitates the development of an environmental risk assessment approach for the resulting impact zone. This study was designed to investigate the impact of low level of dilution (<10) on the natural attenuation processes of distribution and degradation within the impact zone. Dilution of the untreated wastewater resulted in increased desorption and corresponding environmental concentrations. The presence/absence of the microbial population in the batches affected the degree of sorption depending on the compound charge (i.e. positive or negative), highlighting an experimental technical bias. The degradation half-lives of acebutolol and diclofenac increased with increasing dilution and resulted in higher environmental persistence. The modelling of the biochemical oxygen demand (BOD) allowed an estimate of the temporal end boundary of the impact zone to be predicted as 24 h. Therefore, it was concluded that most of the investigated compounds would persist beyond the end of the impact zone as defined by the return to environmental BOD concentrations. It is proposed that, within environmental risk assessment protocols, the impact zone should be considered as a semi-natural wastewater treatment area in such a way to allow the estimate of environmental concentrations of pharmaceuticals beyond its end.

Keywords

Pharmaceuticals; wastewater; biodegradation; dilution; risk; assessment

1. Introduction

Wastewater management is an acute challenge in low and low-middle income countries (LLMICs) where increased volumes of wastewater generated by rapid urbanisation have not been matched by upgrades of sewerage infrastructures resulting in direct discharge of untreated wastewater (DDUW) to surface waters (Malik et al., 2015; Nansubuga et al., 2016). This results in serious pollution of a waterway to such a downstream distance that a combination of dilution and biogeochemical processes such as degradation, volatilisation and sorption render the anthropogenic discharges non-toxic. This area downstream of the DDUW has been defined as an "impact zone", beginning at the DDUW entry point and ending where easily measurable determinants such as biochemical oxygen demand (BOD), ammonia, and dissolved oxygen levels, critical to ecological health, have returned to environmental background concentrations (A.I.S.E./CESIO, 1995; Bagnis et al., 2018a). Whilst DDUW are known to contain high nutrient concentrations and BOD, the presence of emerging contaminants such as active pharmaceuticals ingredients (APIs) is of increasing concern (Keller et al., 2014). This applies particularly to LLMICs where from a pharmaceutical point of view, LLMICs are also experiencing an increase in consumption of medicines, as well as relocation of pharmaceutical production plants to these regions (Kookana et al., 2014).

The impact zone of DDUW has been linked to higher environmental concentrations of APIs than in high income countries with more advanced wastewater treatment plants (Kookana et al., 2014; Malik et al., 2015) and API concentrations up to mg L^{-1} have been detected in impact zones of LLMICs from Africa to Asia (Ashfaq et al., 2017; Madikizela et al., 2017; Ngumba et al., 2016; Shimizu et al., 2013).

To regulate any discharge effectively, it is necessary to be able to define impact zones in order to minimise their extent and plan future wastewater treatment requirements. Furthermore it is necessary to fully understand the fate of chemicals within an impact zone as they are unlikely to behave the same as BOD or ammonia, often used to define such zones. Furthermore, current environmental risk assessment (ERA) guidance for APIs has been developed assuming that sewage is subject to treatment prior to discharge (EMA, 2006), and thus, given that the DDUW does not comply with this assumption, the environmental conditions in the impact zone require a dedicated ERA approach (Bagnis et al., 2018a). This can only be achieved by understanding the mechanisms of natural attenuation that APIs undergo, such as sorption and biodegradation, and the resulting degree of exposure within the impact zone boundaries and beyond.

Additionally, an important mechanism related to the natural attenuation of chemicals in wastewater is dilution (Keller et al., 2014). Many environmental risk assessment and management strategies are overly reliant on the paradigm that dilution reduces a chemical's concentration such that it positively contributes to the reduction of environmental risk in a directly proportional way. In the current ERA protocol, the default ratio of river flow to treated wastewater is set to 10 (EMA, 2006; European Comission Joint Research Centre, 2003). However, despite the conservative nature of the ERA protocol, this dilution factor is overestimated in at least 53 countries, many of which are LLMICs severely affected by DDUW (Keller et al., 2014). Such an assumption may therefore be resulting in under estimates of downstream concentrations of chemicals and given the fact that chemicals are not actually transformed by dilution, this premise is often criticized (Van Breukelen, 2007).

78 A highly important factor often overlooked are the questions as to what the impacts of
79 dilution are on other key processes, such as sorption and biodegradation, which are currently
80 poorly understood but are critical to the derivation of a scientifically robust risk assessment
81 or for use within the development of water quality models (Bagnis et al., 2018a; Hajj-
82 Mohamad et al., 2017).

83 The practicalities of measuring APIs in impact zones of LLMICs with substantial inputs of
84 untreated wastewater are highly challenging. The number of environmental variables are
85 high and sample collection, preservation and analysis is often not possible locally. Therefore,
86 it is necessary to develop and adopt laboratory simulations which provide the opportunity to
87 control important variables such as wastewater quality and characteristics, dilution and API
88 concentration and thus provides the ability to generate vitally important fate data upon
89 which to base future risk assessment approaches.

90 This study focuses on the fate and behaviour of APIs within a simulated impact zone through
91 the recreation of conditions of dissolved organic carbon – DOC (considered a surrogate of
92 BOD), suspended solids and microbiological inoculums on a laboratory scale. Additionally, it
93 investigates the effects of dilution on the main natural attenuation processes controlling the
94 concentration of APIs in the impact zone and beyond, i.e. distribution and biodegradation.
95 Given that there is the potential for some APIs to persist beyond the impact zone defined by
96 parameters such as BOD, for example, owing to either high concentrations discharged or
97 being more recalcitrant than the observed BOD, it is essential to be able to quantify the
98 extent as part of any risk assessment procedure. The overarching aim is to contribute to the
99 development of an approach of environmental risk assessment for APIs within and beyond

the impact zone generated by DDUW. Further contributing to the novelty of this research is the development of a simple model based on simulated BOD concentrations (derived from experimentally derived DOC values) to predict API concentrations outside of the BOD derived impact zone, leading to a draft protocol for the assessment of the environmental risk posed by APIs in LLMICs with limited or poor wastewater treatment.

2. Materials and methods

All glassware and plasticware were cleaned prior to use (2 % v/v Decon, ≥ 24 h; 10 % v/v HCl, ≥ 24 h) and rinsed with ultra-high purity water (UHP) with resistivity above 18.2 MΩ cm (Merck Millipore).

2.1. Active pharmaceutical ingredients

The APIs chosen for this study were the neutral acetaminophen (ACT), carbamazepine (CBZ), and nevirapine (NVR), the acidic diclofenac (DCF) and valsartan (VLS), and the basic acebutolol (ACE) and amitriptyline (AMI). These compounds represent APIs with different structure and functionalities (neutral, acidic and basic) which are commonly consumed in LLMICs (Bagnis et al., 2018a). The chemicals were purchased at the highest purity available, either from Sigma-Aldrich (acebutolol hydrochloride, amitriptyline hydrochloride, nevirapine, valsartan, and acetaminophen) or Fisher Scientific (carbamazepine and diclofenac sodium salt).

2.2. Synthetic wastewater

Wastewater composition varies considerably according to both time and location (Tchobanoglous et al., 2003), presenting challenges in its standardisation for experiments (Boeije et al., 1999). Therefore, a surrogate of synthetic wastewater is needed to achieve experimental reproducibility as well as to ensure there are no background API concentrations present. Also, in order to perform an ERA for chemicals, some form of standardization is needed to facilitate inter- and intra-lab replication. Therefore, a synthetic medium is a logical starting point for such complex studies (Bagnis et al., 2018a). The experimental design was informed by OECD standards tests for chemicals in the environment, which are also standardized in some respect, and was optimised by a through critical evaluation of synthetic wastewaters reported in the literature (Bagnis et al., 2018a; O'Flaherty et al., 2013). Thus, a synthetic wastewater (SW) was prepared to achieve controlled and reproducible experimental conditions (Boeije et al., 1999). In this study, sodium azide (NaN_3) was added to sterilized batches, whilst inoculum (5 mL; consisting of fresh wastewater influent collected at the local wastewater treatment plant of South West Water, Plymouth, England, UK) was added to all other batches (OECD, 1992). The batches containing the inoculum were pre-conditioned for 3 days before the experiments began (OECD, 1992).

2.3. Experimental approach

Dilution factors (DF) of 1, 2, 4 and 10 for untreated SW were prepared in triplicate and a control (500 mL) produced for each DF (Figure 1). The SW was buffered at pH 7.5, which is typical for both wastewater and receiving waters. Inoculum was added to each replicate and left for 72 h before addition of 7 APIs, each to a final concentration of 100 μg L^{-1} as a

representative concentration for impact zones of LLIMCs (K'oreje et al., 2016, 2012; Madikizela et al., 2017; Ngumba et al., 2016). The flasks were wrapped in aluminium foil to avoid photodegradation and continuously stirred. The temperature was 21 °C during the experimental period. After an equilibration time of approximately 4 hours, batches were diluted with UHP at a DF of 1, 2, 4, and 10. Samples were collected at several time points over 50 days for API and DOC determination. An identical abiotic experiment was performed for DF 1 and 2 only, and run for 20 days to determine the sorption component only and/or hydrolysis in order to quantify loss through biodegradation (Figure 1). This second batch was kept sterile by adding sodium azide (0.02%).

2.4. Analytical procedures

Samples were collected in 15 mL centrifuge tubes (Fisher Scientific) and centrifuged for 10 minutes at 4000 rpm. The supernatant was filtered through 0.7 µm GF/F filters (Whatman) in order to allow for solid phase extraction (SPE) and preconcentration without interference from suspended solids present at high levels in the simulated untreated wastewater. For analysis of DOC, an aliquot was diluted 20 times (owing to the high levels present) acidified (HCl, 6 M) and frozen at -20 °C for preservation prior to analysis (Badr et al., 2003).

Concentrations of APIs were measured according to Bagnis et al. (2018). Briefly, aqueous samples were passed through a SPE cartridge for sample clean-up and to optimise analyte resolution, mass detection and quantification. The SPE cartridges (OASIS HLB, 200 mg polymeric sorbent; 6 mL barrel volume; Waters, UK) were activated with 5 mL of methanol (Thermo Fisher Scientific, Optima LC/MS) and 5 mL of UHP. A 5 mL sample aliquot was then loaded onto the cartridge at a flow rate of 3 mL min^{-1}, followed by 1 mL of UHP. The APIs

were eluted with 5 mL of methanol amended with formic acid (2 %). The eluent was evaporated to dryness under a gentle stream of nitrogen gas then reconstituted with 1 ml of methanol : UHP at a 1 : 10 ratio.

Chromatographic separations were measured by high performance liquid chromatography (HPLC) coupled with a high-resolution mass spectrometry (HR-MS) orbitrap-based system (Thermo Scientific), using a reversed phase column (XBridge BEH C18 2.5 µm 2.1 x 50 mm Column XP, Waters) maintained at 50 ^{o}C. A gradient elution programme was employed, starting at 100 % UHP amended with 0.1 % formic acid, progressing to 100 % methanol after 5.5 minutes. The parameters used for high resolution mass spectrometric detection are described in Bagnis et al. (2018).

The dissolved organic carbon (DOC) analyses were performed using high-temperature catalytic oxidation (TOC-5000A - Shimadzu) according to the method of Badr et al. (2003).

2.5. Calculations

Biodegradation rates were assumed to follow first-order kinetics (Schwarzenbach et al., 2003). The 50 % dissipation time (DT_{50}) was modelled as a surrogate measure of the biodegradation rate according to Equation 1:

$$DT_{50} = \frac{0.5IC - I}{K} \quad (1)$$

Where I is the intercept and IC is the initial concentration after dilution in µg L^{-1} and K is the slope of the equation of the line obtained from the linear trend line of the biodegradation starting at the first sample taken after dilution; the dissipation time is expressed in days.

181 BOD was not directly determined owing to practicalities of sample size and time constraints.
182 However, reliable datasets are available that provide a strong correlation between measured
183 DOC and BOD for untreated municipal wastewater (Comber et al., 2018; Kwak et al., 2013).
184 Based on data from over 100 UK wastewater treatment works crude sewage the following
185 relationship was derived from a DOC vs BOD correlation of R^2 = 0.86 (Comber et al., 2018):

$$BOD = \frac{DOC+9.9851}{0.2876} \quad (2)$$

186 # 3. Results and discussion

187 ## 3.1. Effects of dilution on natural attenuation

188 Three out of the seven APIs investigated, namely ACE, DCF and ACT showed effective
189 biodegradation within the simulated impact zone. The biodegradation rate of ACE and DCF
190 was strongly affected by the dilution, showing an overall increase of persistence (longer half-
191 life) at increased dilution. This inverse correlation is well described by the half-life dissipation
192 time (DT_{50}) of DCF and ACE (Table 1).

193 ACE biodegraded over fifteen days then reached a pseudo-plateau with a much slower rate
194 of biodegradation (Figure 2A). The removal rate at no dilution (DF 1) was approximately 78 %
195 after 14 days. This was similar to Lin et al. (2010) who ascribed the disappearance of the
196 compound to sorption processes. However, based on the tests carried out here and
197 examining the data for the abiotic tests here (Figure 2B), it appears that the sorption of ACE
198 was much less significant than biodegradation (Lin et al., 2010). Lin et al. (2010) suggested
199 that their results were most likely due to experimental artefacts which might explain such
200 difference between the two studies. Nonetheless, the progressive dilution from DF 1 to 10

reduced the original concentration of the API, but also the biodegradation rate (Figure2A) and therefore the removal of ACE from the solution. In this case, the progressive increase of dilution concurrently reduced the degrading microbial biomass present in the system, which might explain the trend. This observation is consistent with previously reported studies for other compounds in continuous input studies (Landa et al., 1994).

In the case of DCF the increasing dilution specifically affected its biodegradation profile. In fact, at zero dilution (DF1) the biodegradation curve of DCF showed immediate removal after addition (Figure 2C), whilst a progressive increase in the lag phase prior to biodegradation was observed at DF 2 and 4, lasting respectively 14 and 28 days. At a dilution factor of 10 the DCF concentration remained constant after dilution, for the duration of the whole test (50 days), thus rendering the compound highly persistent. It is worth noting that, although DCF is generally described as fairly biodegradable in wastewater treatment works (Joss et al., 2006; Kruglova et al., 2014), in soils (Al-Rajab et al., 2010) and in aqueous environments (Jiskra and Hollender, 2008; Poirier-Larabie et al., 2016), different environmental conditions can lead to varying degrees of persistence (Baena-Nogueras et al., 2017).

These data strongly suggest that while dilution decreases the concentration of a chemical in the solution, it can increase its persistence. The latter is most likely caused by the concomitant dilution of the bacterial community and the substrate concentration represented by increasing lag phase of acclimation of the bacteria responsible for the contaminant biodegradation (Maier et al., 2009; Swinnen et al., 2004). Such a lag phase is a measure of the resilience of the degrader to such an environmental stress before returning to the conditions necessary to catabolise the contaminant (Ramadan et al., 1990).

223 Furthermore, it has been shown that such a dilution effect might be related to the dilution of
224 other wastewater components such as salts, which indirectly affect the species lag phase
225 duration prior to degradation of the contaminant (Robinson et al., 2001). The reduced
226 removal from dilution of wastewater influent in a wastewater treatment plant has been
227 reported (Joss et al., 2006) and is considered as a factor to be avoided to increase effluent
228 treatment efficiency.

229 The disappearance of ACT in the abiotic batch was extensive, being 40-50 % within 20 days
230 (Figure2F), but the degradation rate was much slower than in the combined sorption-
231 biodegradation batch (Figure 2E), where removal by biodegradation occurred in a few hours
232 (< 12 h). As such, dilution did not visibly influence the biodegradation rate of ACT since the
233 latter was faster than the experimental time designed for batch equilibration. In the study of
234 Lin et al. (2010) consumption of 50 µg L^{-1} was completed with half-life (DT_{50}) of 2.1 days,
235 compared with 3 days for Yamamoto et al. (2009), and 38 days for Baena-Nogueras et al.
236 (2017).

237 The slower disappearance rate observed in such experiments could be explained by the use
238 of river water, which has a lower dissolved organic matter (DOM) concentration and lower
239 microbial biomass as well as different bacterial communities than untreated wastewater.
240 Such differences highlight the likely variable rates of biodegradation for APIs entering the
241 environment in wastewater as treated effluent or untreated sewage (Tappin et al., 2014,
242 2012).

243 CBZ, NVR, VLS, and AMI were persistent over the 50 days of the experiment (Figure 3A, C, E,
244 G), consistent with their known metabolic stability (Nassar et al., 2004; Siddiqui et al., 2011).

245 The only effective natural attenuation processes occurring in the impact zone for these APIs
246 were sorption and dilution.

247 The neutral species NVR and CBZ have a similar chemical structure which can explain their
248 comparable behaviour (Bagnis et al., 2018b). Their sorption was moderate (10-20 %),
249 consistent with their similar lipophilicity (log K_{ow} 2.7 and 2.5 for NVR and CBZ, respectively);
250 no further losses were observed in either the biotic and abiotic batches (Figure 3A, B). Whilst
251 sorption and dilution were the main factors controlling attenuation of CBZ and NVR in both
252 biotic and abiotic batches. A positive deviation from the expected concentration occurred
253 after dilution suggesting desorption.

254 The high frequency of detection of NVR in the environment (K'oreje et al., 2016; Madikizela
255 et al., 2017; Ngumba et al., 2016) has been attributed to both its widespread use and to poor
256 removal efficiency in wastewater treatment plants (Madikizela et al., 2017). The work of
257 Vankova et al.(2010) provides the only available information on the biodegradability of NVR
258 and the results of this study were consistent with that study, which suggested high
259 recalcitrance in wastewater treatment works. Due to the almost identical behaviour of CBZ,
260 further investigation to evaluate its applicability as a possible marker for sewage
261 contamination (Lim et al., 2017) is recommended, especially targeted to African countries
262 where it is widely consumed (Madikizela et al., 2017).

263 Little information is available about the natural attenuation of VLS (Bergheim et al., 2014;
264 Mandaric et al., 2019). The distribution of ionisable APIs is controlled by both lipophilicity and
265 charge, expressed by the ionization constant (pKa). In general, the concentration values for
266 the different dilutions, reported for the ionisable compounds used in this study,

corresponded to the expected calculated arithmetical value plus desorption, when the latter occurred, in accordance with the compound lipophilicity and speciation (Bagnis et al., 2018a). The results for VLS contrasted with reported data, which described a relatively fast biodegradation with consequent detection of transformation products such as valsartan acid, de-alkylated valsartan and amino-valsartan (Helbling et al., 2010; Kern et al., 2010). These differences highlight the variability in behaviour of this API with the experimental conditions employed. While only a few studies on the persistence of AMI have been documented, these report high persistence in both water (Baena-Nogueras et al., 2017) and agricultural soils (Li et al., 2013), in agreement with the data from this study (Figure 3G).

It is worth noting that the results of this work showed a different degree of sorption between the biotic and the abiotic experiments (Figure 2 and Figure 3). For instance, the anionic VLS showed approximately 20 % of sorption in the abiotic experiment compared with no sorption under biotic conditions (Figure 3E and F). The same behaviour was observed for the negatively charged DCF (Figure 2C and D). In contrast, the positively charged AMI had lower sorption under abiotic conditions (Figure 3G and H). In fact, at DF 1, 80 % the compound was adsorbed in the biotic batch as opposed to 50 % in the abiotic one. Such behaviour has been ascribed to the difference of specific surface area for ionic exchange determined by the presence/absence of a bacterial biofilm on the sorbent surface (Carlson and Silverstein, 1998; Headley et al., 1998; Wunder et al., 2011). The bacterial extracellular polymeric substances are composed of anionic (e.g. —COO^-, —SH^-, —SO_4^-, HPO_4^-), cationic (e.g. —NH_3^+), and apolar (e.g. aromatic) functional groups, which at the experimental pH (7.5) are predominantly negatively charged (Wunder et al., 2011). This translates to additional surface sites for exchange of positively charged compounds and the repulsion of negatively charged ones;

290 thus, respectively increasing and decreasing sorption. Such differences between the
291 environmentally more realistic biotic system and the artificial abiotic system highlights a
292 technical bias in the experimental evaluation of the distribution of ionisable chemicals, such
293 as APIs.

294 3.2. APIs persistence through the impact zone

295 Data from the experiments was used to model the environmental concentrations of APIs
296 within and beyond the impact zone and to determine its extent. The end of the impact zone
297 is defined as the point at which BOD reaches background environmental levels (< 1 – 8 mg L^{-1})
298 (Schwarzenbach et al., 2003). Therefore, the experimental DOC was measured and used in
299 the mathematical model (2) derived from the linear correlation of DOC and BOD (R^2=0.86)
300 obtained from a large set of experimental data for untreated wastewater (Kwak et al., 2013).
301 Through this correlation it was possible to estimate the temporal end boundary of the impact
302 zone.

303 According to the model, BOD decreased most rapidly in the first day (Figure 4), achieving a
304 plateau which constitutes the recalcitrant or slowly degrading organic load present in the
305 mixture (dashed red line in Figure 4) (Dignac et al., 2000). The percentage of refractory DOC
306 in wastewater can be highly variable, but roughly comprised 10-30 % of the initial amount
307 (Dignac et al., 2000; Reynolds, 2002), consistent with the generated data (Figure 4). The
308 recalcitrant DOM could not be excluded from the simple modelling approach adopted in this
309 study and therefore the plateau was considered as the lowest level of observable BOD, and
310 consequently marked the end of the impact zone (A.I.S.E./CESIO, 1995). The end of the
311 impact zone was calculated to be reached in approximately 24 hours irrespective of dilution.

312 The modelled data suggested that six of the seven APIs investigated in this study would
313 persist through the impact zone and occur at the initial observed concentration beyond its
314 end boundary. Furthermore, although ACT was quickly biodegraded, the high rate of input
315 and patterns of use could lead to pseudo-persistence in the natural environment, leading to
316 the occurrence in concerning concentrations (> 0.01 μg L^{-1}) beyond the impact zone end
317 boundary.

318 Nonetheless, it must be highlighted that uncertainties are likely to be associated with both
319 the model applied for the calculation of BOD and the calculation of the 24h estimate when
320 applied to a real environmental setting. These uncertainties are related to the environmental
321 complexity of each individual scenario which translate in likely varying DOM composition and
322 consequently variable rates of consumption; therefore, further studies are necessary for the
323 development of a more accurate model.

324 ### 3.3. Implications for an environmental risk assessment approach

325 This work provides novel, robust data that could inform for the future development of an ERA
326 approach for the impact zone with regard to APIs. The data presented here suggests that
327 natural attenuation in the impact zone receiving untreated or poorly-treated wastewaters
328 might not be enough to significantly restrict the environmental burden of APIs within its
329 boundaries. In fact, even at the highest rate of biodegradation, the initial concentration of
330 APIs in the impact zone could be expected beyond its end boundary.

331 According to this investigation, while low levels of dilution (< 10) reduce the API
332 concentration, the rate of biodegradation also slows increasing the risk of environmental
333 persistence and likely occurrence of the API beyond the impact zone boundary. The dilution
334 level of down-the-drain chemicals in surface waters is highly dependent on local hydrological
335 conditions, such as seasonal runoff variability and so this must also be considered in the
336 evaluation of the risk (Keller et al., 2014). Therefore, further studies are necessary to evaluate
337 this important factor contributing to the environmental exposure of APIs in the impact zone.

338 Based on the data presented here, it is possible to propose a possible preliminary ERA
339 approach. In phase 1 of the European ERA a predicted environmental concentration (PEC)
340 above 0.01 μg L^{-1} triggers a tier of tests to refine the understanding of the APIs environmental
341 fate (EMA, 2006). Such calculations assume no biodegradation or loss (to sludge or
342 atmosphere) within the wastewater treatment plant. In the case of DDUW the API
343 concentrations would likely exceed such exposure threshold and as a consequence the
344 development of a dedicated phase with the risk assessment is proposed (Figure 5; proposed
345 as phase 2.2). It does not aim to reproduce the current ERA for APIs but is strongly informed
346 by such protocols and compliments it to take account of DDUW. The new phase of the ERA
347 would be parallel to the current phase 2, renamed in Figure 5 as phase 2.1, for the evaluation
348 of the PEC for APIs beyond the impact zone generated by the DDUW. This phase is divided in
349 two parts, tier A and B, as for phase 2.1. Tier A is based on the modelling methodology used
350 in this study and therefore constrains the impact zone at 24 h. In accordance with the
351 guidelines for the ERA, the experimental studies necessary to evaluate environmental fate
352 should be based on protocols issued by the European Commission, the Organization for
353 Economic Co-operation and Development (OECD) or the International Organization for

354 Standardization (ISO) (EMA, 2006). The PEC beyond the impact zone would therefore be used
355 to evaluate the environmental risk using the PNEC calculated as from the actual protocol
356 (EMA, 2006).

357 If a risk is still evident further refinement of the PEC would be necessary in tier B, taking into
358 account parent compounds PEC and PNEC as well as the most relevant metabolic fraction (≥
359 10 % of amount excreted) (EMA, 2006). In this case, an appropriate model should be
360 developed for the impact zone in the same fashion as SimpleTreat model in EUSES (EMA,
361 2006).

362 The approach proposed here would quantify and manage the risk posed by the
363 environmental occurrence of APIs to LLMICs where dilution occurs at factors below 10, such
364 is the case of 53 countries worldwide.

365 4. Conclusions

366 According to the results of this investigation, the dilution significantly affects the
367 biodegradation rate of DCF and ACE and this might be true for other APIs. ACT instead
368 behaves consistently with previously reported data and it is quickly biodegraded in the
369 wastewater solution regardless the degree of dilution. The other compounds here
370 investigated show high persistence along the experimental timescale. The temporal
371 modelling of the extent of the impact zone allow an estimate of its end at about 24 hours
372 after discharge in the environment, without influence by dilution. The model applied to the
373 APIs natural attenuation data of this study shows a persistence beyond the end of the impact

zone. Thus, an ERA approach is proposed, considering the impact zone as a semi-natural wastewater treatment area. The extent of the zone may be modelled with the aim of estimating the APIs concentrations beyond its end, after which the traditional ERA protocol can be applied.

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